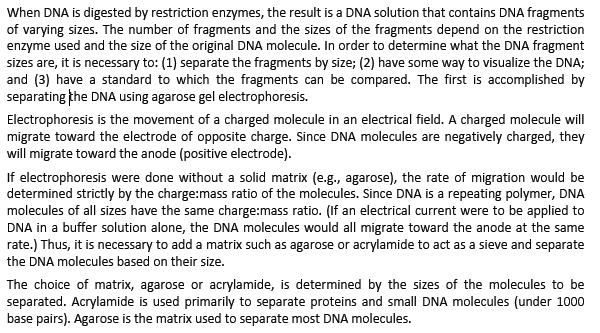
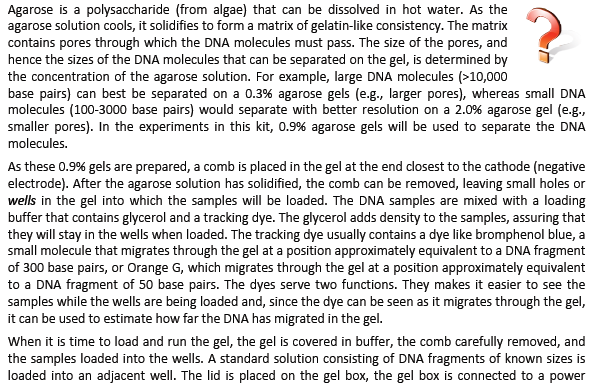
Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Period: \_\_\_\_\_\_

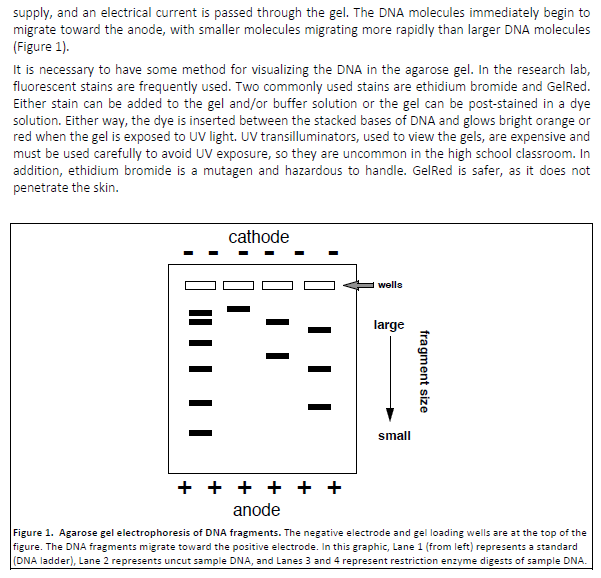
**Gel Electrophoresis Lab**

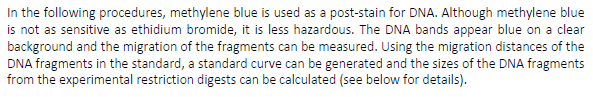
Ms. Ottolini, AP Biology

**Background Information:** *From the Fralin Life Science Institute at Virginia Tech*









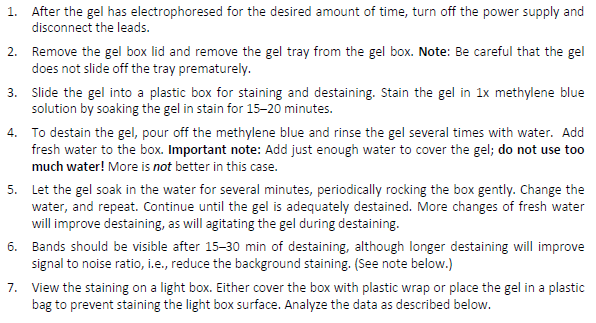
**Procedure for Running the Gel**

1. Use the micropipette to load the wells of the gel with the appropriate samples. Load 10 microliters of sample into each well with the fixed pipette (green) and yellow tip. Your teacher will give you microcentrifuge tubes with the correct amount of each sample.

|  |  |  |
| --- | --- | --- |
| **Well/ Lane** | **Sample** | **Microcentrifuge Tube Color** |
| 1 | DNA Ladder | Orange |
| 2 | pHokie plasmid cut with EcoRI (a restriction enzyme) | Yellow |
| 3 | pHokie plasmid cut with PstI | Green |
| 4 | pHokie plasmid cut with EcoRI and PstI | Blue |
| 5 | “Crime Scene Sample” (what’s in it?) | Purple |

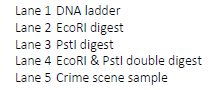
1. Place the lid on the gel box.
2. Connect the leads to the power supply, red to red and black to black.
3. Turn on the power supply at a voltage of 150 volts. It should take about 25-30 minutes to run the gel. Run the gel until the dye front nears the bottom of the gel.

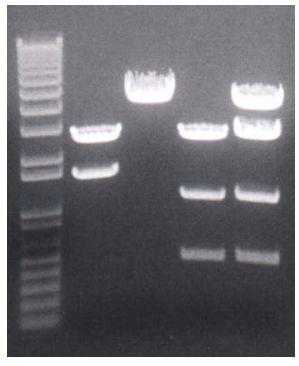
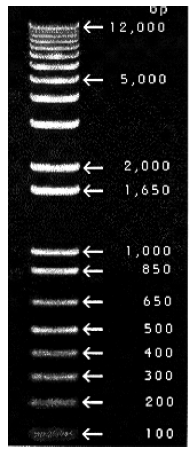
**Procedure for Staining the Gel**

****

**Results and Data Analysis:**

Sample results are given in the picture below. The following samples are found in each lane.





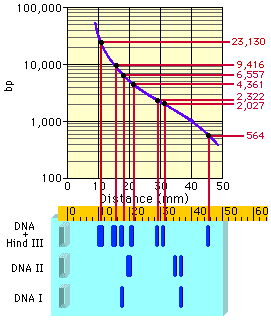
Measure the distance of the DNA ladder fragments (left picture and first lane of right picture) from the top (well end) of the gel. Create a chart comparing the fragment sizes and distance migrated.

|  |  |
| --- | --- |
| **DNA Ladder Fragment Sizes (in base pairs)** | **Distance Migrated (cm)** |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |

Using Microsoft Excel, create a graph of your DNA ladder data by following the steps below…

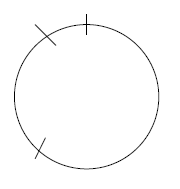
1. Open Microsoft Excel.
2. Enter all the data, keeping the migration distance in the first column and the size of molecules in the second column, as shown by the above example.
3. Select the entire data and click “Insert” and “Scatter” (make sure you choose the ones with the dots connected)
4. In order change the vertical scale (Y axis) to logarithmic scale, right-click on the Y axis on your graph.
5. Choose Format Axis, open Axis Options, and check the box for Logarithmic Scale. Change the Minor Tick Mark Type to Outside.
6. In order to see more divisions on your horizontal scale (X axis, right-click on the X axis on your graph and change the Minor Tick Mark Type to Outside.

After making your graph, you need to use your graph / line connecting the dots to estimate the fragment size based on the distance migrated for the DNA fragments in lanes 2-5 (see below for illustration of method). Record your measurements of the distance migrated and your estimates of the fragment size in the table given below.



|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Lane 2** | | **Lane 3** | | **Lane 4** | | **Lane 5** | |
| **Fragment # (top to bottom)** | **Fragment Size (in base pairs)** | **Distance Migrated (cm)** | **Fragment Size (in base pairs)** | **Distance Migrated (cm)** | **Fragment Size (in base pairs)** | **Distance Migrated (cm)** | **Fragment Size (in base pairs)** | **Distance Migrated (cm)** |
| 1 |  |  |  |  |  |  |  |  |
| 2 |  |  |  |  |  |  |  |  |
| 3 |  |  |  |  |  |  |  |  |
| 4 |  |  |  |  |  |  |  |  |

Use the fragment sizes from lanes 2-4 to map the pHokie plasmid shown below by identifying the cut sites for the enzymes EcoRI and PstI. Note: the total size of the plasmid is approximately 5,000 base pairs.



**Revisions for your Online Lab Portfolio:** If you choose to use this lab for your portfolio, please note the following revisions…

1. You will not create a purpose or hypothesis section for this lab.
2. You will not include a methods summary chart. Instead, your “methods” section will include the following information:

* An explanation of why an electrical current is run through the gel
* An explanation of why/how DNA fragments separate by size in the gel
* An explanation of why restriction enzymes are used in gel electrophoresis

1. Your results section will include both charts given in this packet, a map of the plasmid, and the Excel graph of your DNA ladder data.
2. Your discussion / conclusion section will include the following information:

* A discussion of how DNA ladder data can be used to determine the size of DNA fragments in gel electrophoresis (Note: you should refer to your Excel graph of your DNA ladder data and the method you used to estimate your fragment sizes)
* A discussion of how you determined the restriction enzyme “cut sites” for your pHokie plasmid map
* An identification of the samples found in the Crime Scene “mixture” (ex: a mixture of the EcoRI digest and the EcoRI / PstI double digest) and an explanation for how you determined this (Note: you should refer to the gel picture on page 4 of this packet)